

Recombinant Afamin/Wnt3a

CODE No.	J2-002
FORM	Liquid
QUANTITY	60 µg/300 µL (Theoretical content of Wnt3a is about 37%.)
DESCRIPTION	Recombinant Wnt3a protein and Afamin protein. The N-terminal PA tagged mouse Wnt3a and the N-terminal Myc tagged human Afamin were co-expressed in CHO-K1 Cells.
MOLECULARWEIGHT	mWnt3a : 41 kDa hAfamin : 70 kDa The molecular weight of protein was calculated from the amino acid sequence.
PURITY	Greater than 90% purity as confirmed on SDS-PAGE by Coomassie brilliant blue staining.
ENDOTOXIN LEVEL	< 1.0 EU/mg by the LAL assay.
FORMULATION	60 µg in 300 µL volume of 20 mM Tris-HCl (pH 7.4), 150 mM NaCl.
STORAGE	Store at -20°C or below.
EXPIRATION	Please see the label of this kit.
DIRECTION for USE	As a canonical Wnt signal agonist.

NOTE

We recommend the addition of albumin to dilution buffer to improve freeze-thaw stability, not diluted without albumin such as PBS only, if diluted product is stored in freeze. Dilutions prepared by the following protocol have been shown to maintain high Wnt3a activity after a freeze-thaw cycle.

1. Prepare PBS containing 0.04% HSA (human serum albumin) or BSA (bovine serum albumin).
2. Dilute Recombinant Afamin/Wnt3a with 0.04% HSA or BSA/PBS to prepare 2-, 10-, and 50-fold dilutions.

Use the prepared dilutions as soon as possible and avoid repeated freeze-thaw cycles.

BACKGROUND

Wnt signaling is known to be involved in early development, maintenance and regeneration of stem cells, and in cancer formation. Wnt signaling has also been found to play an important role in the growth and maintenance of these processes. In particular, Wnt3a has been revealed to be an essential niche component for maintaining the proliferation of Lgr5-positive stem cells in intestinal epithelial cells and is used for the production of various digestive organoids such as the small intestine, large intestine, stomach, pancreas and liver. Although Wnt3a has been conventionally used for the culture of gut organoids, it is a fat-soluble protein, so it forms aggregates in serum-free medium and cannot exert its activity sufficiently. In 2016, Mihara *et al.* found that high Wnt3a activity can be maintained by forming a complex with Wnt3a by Afamin, which is one of the components of serum. In addition, by using Afamin and Wnt3a complex for organoid culture, long-term culture of organoid becomes possible. This new medium will result in optimal success for your organoid experiments.

REFERENCES

- 1) E. Mihara, *et al.*, Active and water-soluble form of lipidated Wnt protein is maintained by a serum glycoprotein afamin/ α -albumin., *eLife* **5** (2016) [PMID: [26902720](#)]
- 2) K. Nanki, *et al.*, Divergent routes toward Wnt and R-spondin niche independency during human gastric carcinogenesis., *Cell* **174** (2018) [PMID: [30096312](#)]
- 3) S. Sugimoto, *et al.*, Reconstruction of the human colon epithelium in vivo., *Cell Stem Cell* **22** (2018) [PMID: [29290616](#)]
- 4) T. Seino, *et al.*, Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression., *Cell Stem Cell* **22** (2018) [PMID: [29337182](#)]
- 5) S. Sugimoto, *et al.*, Organoid Derivation and Orthotopic Xenotransplantation for Studying Human Intestinal Stem Cell Dynamics., *Methods Mol Biol.* **2171** (2020) [PMID: [32705652](#)]
- 6) K. Nanki, *et al.*, Somatic inflammatory gene mutations in human ulcerative colitis epithelium., *Nature* **577** (2020) [PMID: [31853059](#)]
- 7) N. Sasaki, *et al.*, Development of a Scalable Coculture System for Gut Anaerobes and Human Colon Epithelium. , *Gastroenterology* **159** (2020) [PMID: [32199883](#)]
- 8) S. Mae, *et al.*, Expansion of Human iPSC-Derived Ureteric Bud Organoids with Repeated Branching Potential. ,*Cell Reports* **32** (2020)[PMID: [32726627](#)]
- 9) Y. Nanki, *et al.*, Patient-derived ovarian cancer organoids capture the genomic profiles of primary tumours applicable for drug sensitivity and resistance testing. ,*Scientific Reports* **28** (2020) [PMID: [32724113](#)]
- 10) K. Miyabayashi, *et al.*, Intraductal transplantation models of human pancreatic ductal adenocarcinoma reveal progressive transition of molecular subtypes. , *Cancer Discov.* **10** (2020)[PMID: [32703770](#)]

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When culturing organoids, or stem cells, or other tissues, if you are to use this product in combination with other factor or factors (hereunder factors), a third party may have a patent on the use or other application of the factors concerned. Regarding to this product, we do not offer any non-infringement warranty when used or otherwise applied in combination with other factors. Therefore, if you intend to use this product in combination with other factors, please check with your organization's division responsible for intellectual property rights or your research agency before using this product.